The sRNA NsiR4 is involved in nitrogen assimilation control in cyanobacteria by targeting glutamine synthetase inactivating factor IF7

Supplementary Material

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Supplementary Materials and Methods

Strains and growth conditions. For the generation of *nsiR4* mutants, we used the glucose-tolerant strain *Synechocystis* 6803 (GT-Kazusa) provided from N. Murata (National Institute for Basic Biology, Okazaki, Japan). Cultivation was performed in Cu^{2+}-free BG11 medium (1) buffered with 20 mM TES, pH 8.0 at 30°C under continuous white light illumination of 50–80 µmol quanta m^{-2} s^{-1} and gentle agitation. Mutant strains were grown in presence of the corresponding antibiotics. To induce nitrogen deficiency, the cells from liquid cultures were harvested through centrifugation (for 5 min at 4.000 rpm and room temperature), resuspended in NO_3^--free BG11 and cultivated further. To re-establish N-replete conditions, NH_4Cl or $NaNO_3$ were added to the respective experiments. To induce the ectopic expression of NsiR4, 2 µM $CuSO_4$ was added to the cultures. *Anabaena* 7120 WT and the mutant strain CSE2 (carrying a streptomycin resistance cartridge within the *ntcA* coding region; (2)) were grown with bubbling (CO_2-enriched air, 1% vol/vol) in BG11 without NO_3^- and supplemented with 6 mM NH_4Cl, 12 mM TES-NaOH (pH 7.5) and 10 mM $NaHCO_3$. For nitrogen step-down experiments, the *Anabaena* cells were collected through filtration, washed and re-suspended in nitrogen-free medium (BG11 without NO_3^-). For long-term growth competition experiments, three independent, exponentially growing cultures of *Synechocystis* 6803 WT and Δ*nsiR4* were diluted to an OD750 of 0.1 and mixed in equal numbers. Cultures were grown in 20 ml BG11 in 100 ml Erlenmeyer flasks with 1 mM NO_{3-} (instead of the usual 17.6 mM). After 3 or 4 days cultures were re-diluted to an OD750 of 0.1 and 25 µl of a 1:1000 dilution were dropped on BG11 agar plates with and without 40 µg/ml kanamycin. Amplification of the *nsiR4* locus was made using primers SyR12_ko_seg_for/rev and genomic DNA of WT and Δ*nsiR4* cultures and a representative, mixed culture after 3, 24 and 45 days.

Genome and promoter analysis. The 70 nt of *Synechocystis* NsiR4 (pos. 1289326 – 1289257, complementary strand) was used as a reference for BlastN searches in the JGI database. However, the identification of an sRNA gene based on sequence alone is not straightforward due to the short length and little sequence conservation. Therefore, the sequences of the resulting hits were extended 100 bp in both directions and further analyzed in multiple alignments using ClustalW (3). The presence of *nsiR4* in a given genome was regarded as positive when the sequence aligned to the corresponding sequence from *Synechocystis* and contained a potential terminator hairpin. Initially, the MEME search tool (4) was used for the comparative analysis of sequences identified upstream of the identified *nsiR4* homologous genes. To verify the activity of the *nsiR4* promoter in *Synechocystis in vivo* a sequence spanning the range between -130 to +49 (respective to the TSS at +1) was fused to *luxAB* reporter genes. The fragment was amplified from the gDNA of *Synechocystis* (for oligonucleotides see **Table S2**), followed by restriction digestion with *Kpn*I and cloning into the promoter-probe vector pILA (5). For mutagenesis of the NtcA motif, the corresponding plasmid was re-amplified using the primers prNtc_mut_fw/rev (**Table S2**). The resulting plasmids, containing either the native or a mutated NsiR4 promoter, were used to transform a *Synechocystis* host strain carrying the *luxCDE* operon, which encodes enzymes for the synthesis of decanal, the substrate for the

luciferase reaction. The selection of the reporter strains and bioluminescence measurements were performed as described (6).

Generation of NsiR4 mutant strains. A schematic presentation of the cloning strategies is shown in **Supplementary Figure S6**. To generate the NsiR4 knockout strain (Δ*nsiR4*), two fragments covering the adjacent genes *sll1697* and *sll1698* were amplified from gDNA using the primer combinations 5'SyR12_for/5'SyR12_BsrGI_rev and 3'SyR12_PstI_for/3'SyR12_rev (**Supplementary Table S2)**. The PCR products were digested with the restriction endonucleases *Bsr*GI and *Pst*I, respectively. A kanamycin resistance cartridge (Km^R) was amplified from the vector pVZ322 using the primers Kan_PstI_for and Kan_BsrGI_rev, and subsequently digested with *Bsr*GI and *Pst*I and ligated to the compatible ends of both fragments using T4 DNA ligase (Thermo Scientific). The resulting construct comprising a Km^R flanked by sequences homologous to the genes *sll1697* and *sll1698* was re-amplified using the primers 5'SyR12_for and 3'SyR12_rev and introduced into the cloning vector pJET1.2. This plasmid was used to transform WT *Synechocystis*. The mutant cells were initially selected on BG11 agar plates (0.9% Kobe I agar, Roth, Germany) supplemented with 10 µg ml^{-1} kanamycin and subsequently grown in the presence of 50 µg ml^{-1} in liquid cultures.

To establish the ectopic expression of *nsiR4*, a self-replicating plasmid carrying the *nsiR4* gene under control of the *petE* promoter, which mediates Cu^{2+}-regulated transcription in *Synechocystis* (7), was prepared. The genomic sequence of *nsiR4* was amplified from *Synechocystis* gDNA using the primers SyR12_EcoRI_for and SyR12_EcoRI_rev. The product was digested with *Eco*RI and introduced into a vector as previously described (8). This plasmid is based on pJET1.2 and contains an *Eco*RI site between the *petE* promoter from *Synechocystis* (ranging from nucleotide -235 to -1 with respect to the TSS at +1), (9) and the oop-terminator. The entire construct was integrated into the *Synechocystis* chromosome via homologous recombination into the *spkA* locus, which is a neutral site in the WT strain used here (8). However, divergent from the initial idea of chromosomal integration we cloned the cassette P*petE*::*nsiR4*::oop into the replicative broad-host vector pVZ322. The construct was re-amplified using the primers spk_km_hindIII_for and spk_km_xhoI_rev, subsequently digested with *Hin*dIII and *Xho*I and introduced into the plasmid pVZ322, digested with the same enzymes (note that the Km^R of pVZ322 was deleted after *Hin*dIII/*Xho*I treatment). The resulting plasmid was transferred into WT *Synechocystis* and Δ*nsiR4* via conjugal transfer from *E. coli* (10), resulting in the strains NsiR4oex (in WT) and Δ*nsiR4*::oex (in Δ*nsiR4*), respectively. The recombinant strains were selected on BG11 agar containing 1 µg ml^{-1} gentamycin and also grown in presence of the same concentration in liquid cultures.

RNA extraction, microarrays and Northern blots. The collection of *Synechocystis* cells and RNA extraction was performed as previously described (6, 11). Prior to the microarray analysis, 10 µg of total RNA were treated with Turbo DNase (Invitrogen) according to the manufacturer's protocol and precipitated with ethanol/sodium acetate. Labeling and hybridization were performed as previously described (12), using 3 µg of RNA for the labeling reaction and 1.65 µg of labeled RNA for the hybridization. For Northern hybridization,

Synechocystis 6803 RNA was separated on denaturing agarose gels and transferred to Hybond-N^+ membranes (Amersham, Germany) through capillary blotting with 20x SSC buffer. The membranes were hybridized with [α-^{32}P]-UTP incorporated single-stranded RNA probes generated through *in vitro* transcription as previously described (13). The signals were detected using a Personal Molecular Imager system (Pharos FX, BIO-RAD, Germany) and analyzed using Quantity One software (BIO-RAD, Germany). RNA from *Anabaena* was isolated using hot phenol (14). Total RNA was separated on urea-acrylamide gels and transferred to Hybond N^+ membranes with 1x TBE buffer in a semi-dry blotter. The membranes were hybridized with oligonucleotides labeled with γ-^{32}P-dATP and polynucleotide kinase (probe for NsiR4) or with probes labeled with γ-^{32}P-dCTP and Ready-to-go DNA labeling kit (Amersham) (probe for 5S rRNA).

Protein extraction and immunoblots. *Synechocystis* and derivative strains were grown in NO_3^--containing, Cu^{2+}-free medium. For analysis of IF accumulation, 2 µM $CuSO_4$ was added to induce transcription from the *petE* promoter eight hours before addition of 10 mM NH_4Cl and 20 mM TES-NaOH (pH 7.5). Samples were taken prior to and in a narrow time series after NH_4^+ addition. Cells from different time points were collected by centrifugation and frozen until protein extraction. Extracts were prepared using glass beads as previously described (15) in 50 mM Hepes-NaOH buffer (pH 7.0), 50 mM KCl, 1 mM EDTA. For Western blot analysis proteins were fractionated on 15% SDS-PAGE and immunoblotted with anti-IF7 (1:2000), anti-IF17 (1:2000) or anti-TrxA (1:3000). Anti-IF7, anti-IF17 and anti-TrxA antisera were obtained from M.I. Muro-Pastor and F.J. Florencio and used as described (16, 17). The ECL Plus immunoblotting system (GE Healthcare) was used to detect the different antigens with anti-rabbit secondary antibodies. Densitometric evaluation was performed with Quantity One software.

Reporter assays for the *in vivo* verification of targets. For the experimental target verification, we used the reporter system described by (18) and the sGFP plasmid pXG-10-SF introduced by (19). The primers used for cloning and the resulting plasmids are given in **Tables S2 and S3**. The entire 5'UTR containing the predicted NsiR4 interaction sequence and a part of the coding region were amplified from gDNA using the primer combinations tv_ssl1911_gifA_for/tv_ssl1911_gifA_rev or tv_ssr1528_fw/tv_ssr1528_rev and which covered ranges from +1 to +123 (*gifA*) or +1 to +119 (*ssr1528*) with respect to the TSS at position +1. The first nucleotide of the *gifA* start codon is at +52, for *ssr1528* at +30. For *gifA* the information about the TSS has been taken from (20), for *ssr1528* it was extracted from (9). The corresponding PCR product was cloned into the vector pXG-10-SF via the endonuclease sites *Nsi*I/*Nhe*I resulting in a translational fusion of the sGFP with a truncated IF7 or Ssr1528 protein. The transcription is mediated by the constitutive promoter $P_{LtetO-1}$. For the preparation of the plasmid establishing $P_{LlacO-1}$-mediated sRNA expression in *E. coli* the *nsiR4* gene was amplified from gDNA using the primers 5_SyR12_long_phos/3_SyR12_xbal, digested with *Xba*I and fused to a plasmid backbone which was amplified from pZE12-*luc* (by using the primer combination PLlacoB/PLacoD) and also digested with *Xba*I.

For the mutagenesis of NsiR4 and the 5'UTRs of *gifA* and *ssr1528*, the plasmids harboring the native versions were re-amplified using the primers SyR12_1911_mut_fwd/ SyR12_1911_mut_rev or SyR12_1528_mut_fwd/ SyR12_1528_mut_rev (for mutating NsiR4 parts interacting with *gifA* and *ssr1528*, respectively) and PXG10_1911_mut_fwd/ PXG10_1911_mut_rev (*gifA*) or PXG10_1528_mut_fwd/ PXG10_1528_mut_rev (*ssr1528*) (for the respective 5'UTRs) and introduced into *E. coli*. Positions for mutations were selected on the basis of lowered hybridization energies predicted by IntaRNA (21) while keeping the secondary structures as calculated with RNApdist (22). For testing various combinations of both plasmids, these were introduced into *E. coli* TOP10 (Invitrogen): e.g. pXG0 + pJV300, pXG10-*gifA* + pJV300/pZE12-NsiR4. The plasmids pJV300 and pXG-0 were used as negative control plasmids. The fluorescence measurement was done as described previously (23).

Supplementary Figures

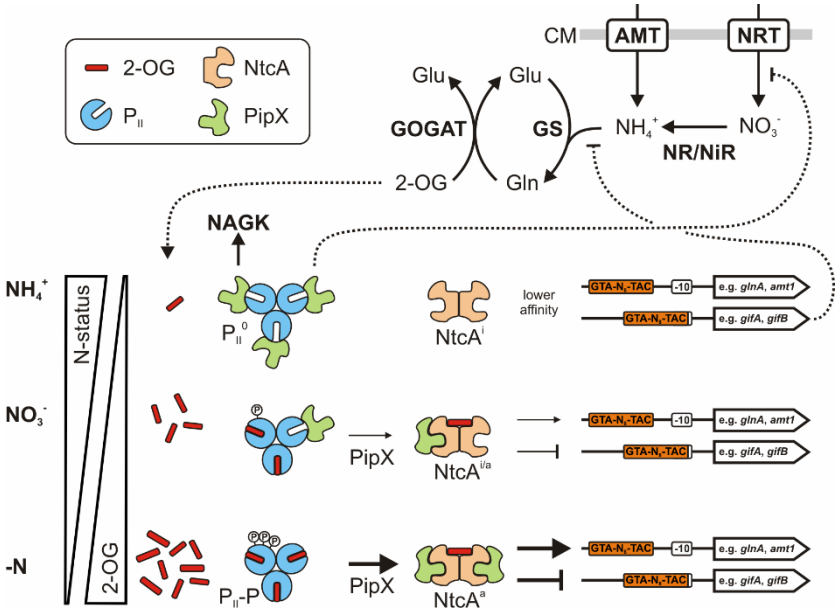

Fig. S1: The nitrogen regulatory network in cyanobacteria. The scheme was prepared based on references (20, 24–27).

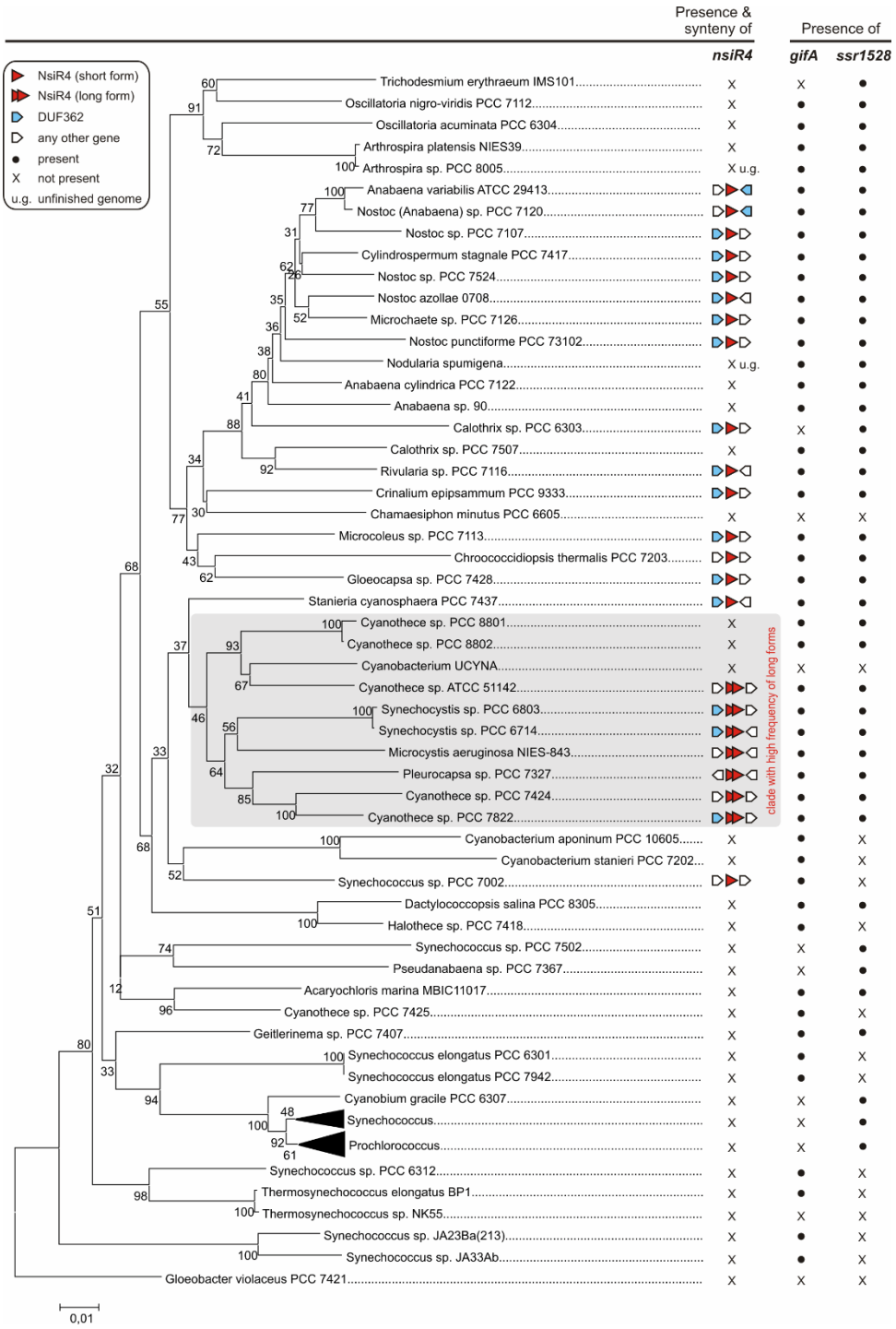

Fig. S2: Presence and synteny of the genomic locus for *nsiR4* among the cyanobacterial phylum in combination with the presence of *gifA* and *ssr1528* homologous genes. The representative phylogenetic tree was generated by using the neighbor joining algorithm based on cyanobacterial 16S rRNA sequences that were extracted from the SILVA database (28). By using BlastN and the JGI database (https://img.jgi.doe.gov/cgi-bin/w/main.cgi) all available genomes were screened for a sequence similar to NsiR4 from *Synechocystis* 6803. In order to sustain clarity, several genomes which also harbor short forms of NsiR4 were not included in the phylogenetic tree (e.g. *Leptolyngbya* strain PCC7376, *Fischerella thermalis* PCC7521). Presence of *gifA* and *ssr1528* genes was analyzed using the BlastP algorithm and the corresponding amino acid sequences from *Synechocystis* 6803 (E-value cutoff of 1e-5).

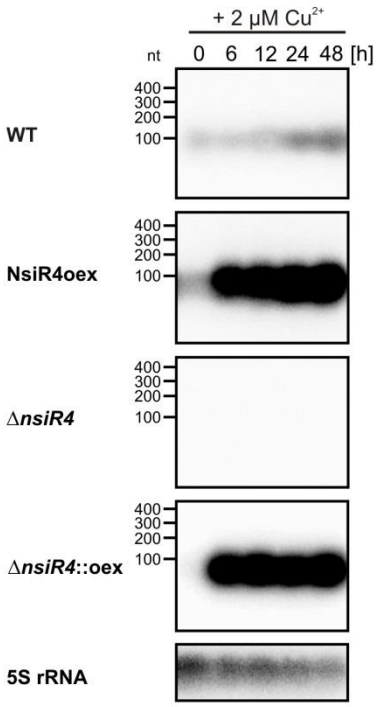

Fig. S3: Verification of the *nsiR4* mutant strains through northern blot analysis. Expression kinetics of NsiR4 was measured in cells grown in the presence of 17.6 mM NO_3^- and after the addition of 2 µM Cu^{2+}. For clarity, only one representative 5S rRNA loading control hybridization for NsiR4oex is shown. WT - *Synechocystis* 6803 wild type, NsiR4oex – WT strain carrying pVZ322-P*petE*::*nsiR4*::oop plasmid (overexpression strain), Δ*nsiR4* - deletion mutant, Δ*nsiR4*::oex - deletion strain in which NsiR4 expression was restored through the pVZ322-P*petE*::*nsiR4*::oop plasmid.

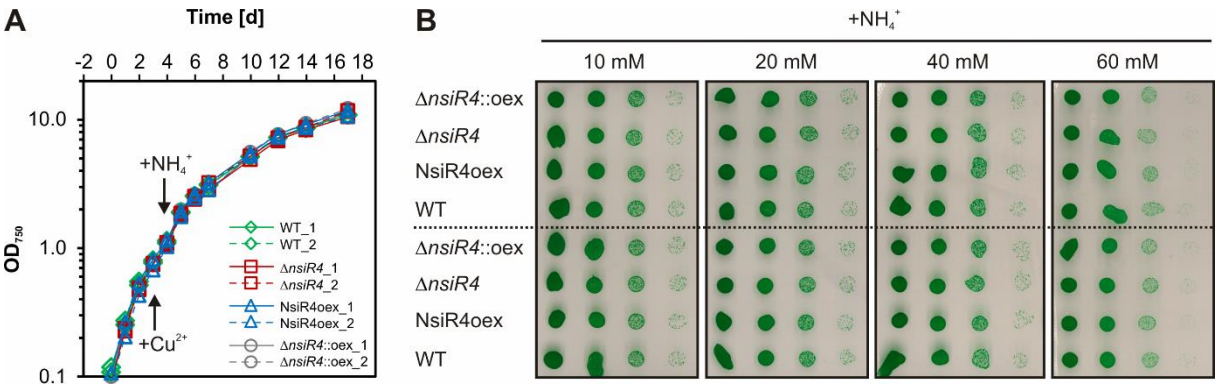

Fig. S4: Growth performance of *nsiR4* mutant strains. A: Growth curve of two replicates per strain. To induce NsiR4 overexpression (in strains NsiR4oex and Δ*nsiR4*::oex) and to maximize the effect on *gifA* repression, 2 µM Cu^{2+} and 10 mM NH_4^+ were added to the cultures (indicated by arrows) that were initially inoculated in copper-free BG11 medium containing 17.6 mM NO_3^-. **B:** Drop dilution assays for two biological replicates and various NH_4^+ concentrations. Cells were grown in the presence of nitrate and the corresponding antibiotics. Cells were washed and resuspended at a concentration of 1 µg chlorophyll *a*/ml. Three 10-fold serial dilutions were prepared and 5 µl of each dilution were plated. All plates were supplemented with 2 µM Cu^{2+}. Photograph was taken after 5 days of growth. WT - *Synechocystis* 6803 wild type, NsiR4oex – WT strain strain carrying pVZ322-P*petE*::*nsiR4*::oop plasmid (overexpression strain), Δ*nsiR4* - deletion mutant, Δ*nsiR4*::oex - deletion strain in which NsiR4 expression was restored by the pVZ322-P*petE*::NsiR4::oop plasmid.

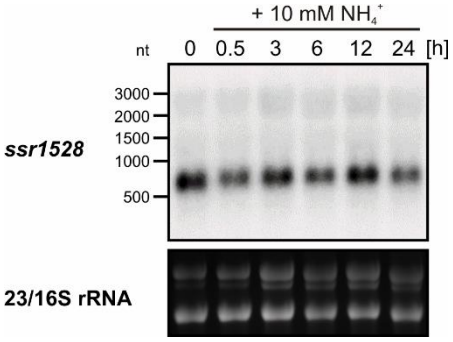

Fig. S5: Expression of *ssr1528* in *Synechocystis* WT after adding 10 mM ammonium. Prior to ammonium addition the strains were pre-cultivated for 6 h in presence of 2 µM Cu^{2+}. Total RNA was extracted, gel-separated, blotted onto Hybond-N^+ nylon membranes and hybridized with specific, ^{32}P-labelled, single-stranded RNA-probes. As loading control a part of the corresponding agarose gel 23S and 16S rRNA is shown.

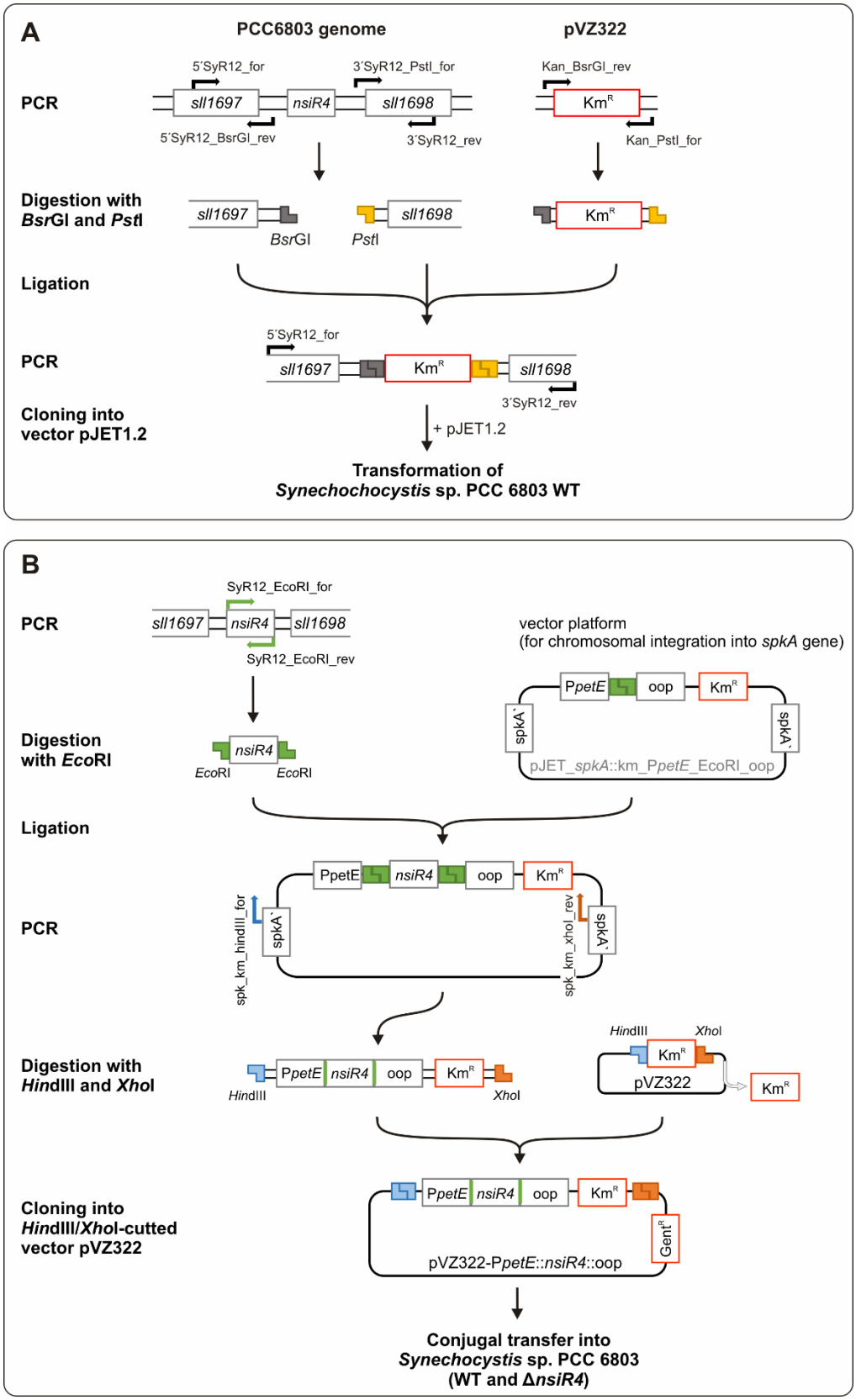

Fig. S6: Schematic view of the cloning strategies. A: The knockout mutant Δ*nsiR4*. **B:** The overexpression strain NsiR4oex and the compensatory strain Δ*nsiR4*::oex.

Supplementary Tables

Table S1: List of genes with a lowered expression level in strain NsiR4oex. Given are log_2 fold changes in the corresponding strains vs. WT. Additionally to the sorted list of fold changes in NsiR4oex, the corresponding log_2 values are also given for the strains Δ*nsiR4* and Δ*nsiR4*::oex. The two genes shown in bold were the only examples for which log_2 fold changes <-1 were obtained in the NsiR4oex strain and for which additionally an opposite change was observed in the Δ*nsiR4* knockout strain.

NsiR4oex	Δ*nsiR4*	Δ*nsiR4*::oex	Locus Tag	Gene Name	Annotation
-1.18	**1.04**	**-0.68**	***ssr1528***	**N/A**	**hypothetical protein**
-1.10	**1.12**	**-0.86**	***ssl1911***	***gifA***	**glutamine synthetase inactivating factor IF7**
-0.69	-0.80	-1.29	*slr0447*	*urtA*	urea transport system substrate-binding protein
-0.69	-0.44	-0.92	*sll0108*	*amt1*	ammonium transporter Amt family
-0.54	-0.43	-0.16	*slr1513*	N/A	hypothetical protein
-0.51	0.05	-0.48	*ssr0692*	N/A	hypothetical protein
-0.49	-0.41	-0.23	*sll0822*	*abrB*	AbrB-like transcriptional regulator
-0.49	-0.31	-0.10	*slr1512*	*sbtA*	sodium-dependent bicarbonate transporter
-0.48	-0.34	0.11	*slr0041*	*cmpB*	bicarbonate transport system permease protein
-0.46	0.38	-0.16	*slr0904*	N/A	magnesium chelatase family protein

Table S2: List of oligonucleotides.

Name of Oligonucleotide	Sequence (in 5' – 3' direction)	Application
Generation of *nsiR4* mutant strains in *Synechocystis* sp. PCC 6803		
5'SyR12_for	CTCCGGTCCCAATCCTACGAAGC	Amplification of sequence flanking *nsiR4* upstream region
5'SyR12_BsrGI_rev	GAATGTACAGGCCGGATCGGTAGGCTTTATGTAG	
3'SyR12_PstI_for	GAACTGCAGCCCATTGCTTCAGTGGCGGCTTTC	Amplification of sequence flanking *nsiR4* downstream region
3'SyR12_rev	GCCGTCAGACCAACGCAGACC	
Kan_PstI_for	GAACTGCAGAATAAAAAACGCCCGGCGGCAACCGAGCGAATCCCGTCAAGTCAGCGTAATGCTC	Amplification kanamycin resistance cassette from pVZ322
Kan_BsrGI_rev	GAATGTACACAAAGCCACGTTGTGTCTCAAAATCTCTG	
SyR12_ko_seg_for	CGTCCCAAATCGAGCAGTGCATG	Verification of Δ*nsiR4* knockout mutants
SyR12_ko_seg_rev	CTAGGGTGTTGCGTTCCACGTTC	
SyR12_EcoRI_for	GAAGAATTCAAGACATAAAGTCAATATCACCCTCCGATTGC	Amplification of *nsiR4* from *Synechocystis* sp. PCC 6803 for generating an NsiR4 expressing plasmid
SyR12_EcoRI_rev	GAAGAATTCGCATGGCAGCTTCTAAAGGACTAATAAACTC	
spk_km_hindIII_for	GAAAAGCTTCATTTCCGACACCGAGAAAACC	Amplification of insert (P*petE*-NsiR4-oop) from shuttle vector pJET_spkA::km_P*petE*_ ecoRI_oop for ligation into pVZ322
spk_km_xhoI_rev	GAACTCGAGTGGATGATGGGGCGATTCAG	
Oligonucleotides used for Northern blots (T7 promoters are underlined)		
fw_pro_ssr1528	GATCGCCGCTGGCATTGATTTTGATGGC	Amplification of PCR template used for *in vitro* transcription generating probes for *ssr1528*
rev_pro_ssr1528	<u>TAATACGACTCACTATAGGG</u>GCGGGAGCGCATGGTATTACTGACCCC	
rev_pro_ssl1911	ATGTCTACTCAACAACAGGCTCGCGCT	Amplification of PCR template used for *in vitro* transcription generating probes for *gifA* (*ssl1911*)
fw_pro_ssl1911	<u>TAATACGACTCACTATAGGG</u>AGCGGCAGCGCGGGACAACATGGA	
5sRNA_for	<u>TAATACGACTCACTATAGG</u>AGAAAGAGGAACTTGGCATCGGAC	Amplification of PCR template used for *in vitro* transcription generating probes for 5S rRNA
5sRNA_rev	GTCATGGAACCACTCCGATCCC	
Oligo probe for NsiR4 (*Anabaena* 7120)	GGTCTGGTTAAGCAATCGGAGGGTAAT	Northern blot for NsiR4 detection in *Anabaena* 7120
7120-rrn5Sa-1	AGTTTTCCTGGTGCCTATG	PCR fragment probe for detection of 5S rRNA in *Anabaena* 7120
7120-rrn5Sa-2	ACCTGGCACCGAGCGATTG	
LuxAB reporter assays		
Syr12-KpnI_fw	GGTACCCCACGTTCAAACACTTTTACATTCG	Amplification of the *nsiR4* promoter from *Synechocystis* 6803 for cloning into pILA reporter-plasmid
Syr12-KpnI_rev	GGTACCGCAATGGGCGACCTCTAGC	
prNtc_mut_fw	CTCAAATAGGCATCATAAAGCCTACCGATC	Mutation of the putative NtcA motif upstream of *nsiR4*
prNtc_mut_rev	GATGCCTATTTGAGGAAAGTTCCCGTAAC	
Target verification in *E. coli* using a Gfp Reporter system (nucleotides underlined and shown in bold refer to the introduced point mutations)		
tv_ssr1528_fw	ATGCATAGTAAAATAACTCGAGGGTAATATTGATCATGG	Amplification of putative target gene-sequence containing the predicted interaction-region with
tv_ssr1528_rev	GCTAGCCTTGGCTTCGGGAATGGCACTG	

tv_ssl1911_gifA_for	ATGCATAGAGGGTAATTAACCAAAACTTTTTTCAG	NsiR4. The sequence consists of the respective 5´UTR and part of the coding region
tv_ssl1911_gifA_rev	GCTAGCGGATTGTTGACGGTTTTTGATGAATTG	
PLlacoB	CGCACTGACCGAATTCATTAA	Amplification of fragment from plasmid pZE12_luc
PLlacoD	GTGCTCAGTATCTTGTTATCCG	
5_SyR12_long_phos	AAGACATAAAGTCAATATCACCCTCC	Amplification of *nsiR4* (long version) for ligation into pZE12-luc; phosphorylated
3_SyR12_xbal	GTTTTTTCTAGATAAAGGACTAATAAACTCTAAAAAGAAAGCC	Reverse primer for the amplification of *nsiR4* for ligation into pZE12-luc
PXG10_1911_mut_rev	TGACGATT**A**CTGAAAAAAGTTTTGGTTAATTAC	Introduction of a point mutation into *gifA* sequence
PXG10_1911_mut_fwd	TTCAG**T**AATCGTCAAGAGGTATTAACTAT	
PXG10_1528_mut_rev	CCATGATCAA**AT**TTACCCTCGAGTTATTTTA	Introduction of a point mutation into *ssr1528* sequence
PXG10_1528_mut_fwd	GTAA**AT**TTGATCATGGCTAATACAACTAAAGGA	
SyR12_1911_mut_rev	CGACCTCTAG**T**AATCGGAGGGTGATATTG	Introduction of compensatory mutation into *nsiR4* sequence (*gifA* mRNA as target)
SyR12_1911_mut_fwd	CGATT**A**CTAGAGGTCGCCCATTGCTT	
SyR12_1528_mut_rev	TGA**AT**TTGACTTTATGTCTTGTGCTCAGT	Introduction compensatory mutation into *nsiR4* sequence (*ssr1528* mRNA as target)
SyR12_1528_mut_fwd	ATAAAGTCAA**AT**TCACCCTCCGATTGCTA	

Table S3: List of plasmids.

Plasmid	Plasmid backbone	Description	Marker	Reference
pJET_spkA::km_P*petE*_ecoRI_oop	pJET1.2	Shuttle vector for insertion of *nsiR4*-sequence. Generation of NsiR4 expressing plasmid	Km^R	This study
pJET-spkA::km_P*petE*_*nsiR4*_oop	pJET1.2	Shuttle vector for re-amplification of cassette P*petE*::*nsiR4*::oop	Km^R	This study
pVZ322-P*petE*_NsiR4_oop_km	pVZ322	Plasmid for copper-inducible NsiR4 expression in *Synechocystis* sp. PCC 6803	Gen^R, Km^R	This study
pJET-*ssl1697*::km::*ssl1698*	pJET1.2	Generation of *nsiR4* knockout strain	Km^R	This study
pILA		Promoter probe vector harbouring the promoterless *luxAB* genes encoding luciferase, contains recombinations sites for integration into the *Synechocystis* chromosome	Km^R, Amp^R	(5)
pILA-P*nsiR4*	pILA	pILA harbouring *luxAB* under control of the *nsiR4* promoter (range between -130 to +49 with respect to the transcriptional start siteTSS at +1 was used)	Km^R, Amp^R	This study
pILA-P*nsiR4*_mut	pILA	same as pILA-P*nsiR4* but carrying a mutated NtcA binding motif	Km^R, Amp^R	This study
pZE12-luc		General expression plasmid	Amp^R	(29)
pJV300	pZE12-luc	Control plasmid, expressing a ~50 nt nonsense transcript derived from rrnB terminator	Amp^R	(30)
pXG0	pZA31-luc	Plasmid expressing luciferase used as a negative control; cell autofluorescence	Cm^R	(18)
pXG10	pXG0	Plasmid for synthesis of translational sfGFP fusions	Cm^R	(19)
pXG10_ssl1911	pXG10-SF	GFP reporter plasmid containing the *gifA* 5'UTR plus the initial part of the coding region	Cm^R	this study
pXG10_ssr1528	pXG10-SF	GFP reporter plasmid containing the *ssr1528* 5'UTR plus initial part of the coding region	Cm^R	this study
pXG10_ssl1911_mut	pXG10-SF	GFP fusion plasmid containing the *gifA* 5'UTR with mutation in the interacting region with NsiR4	Cm^R	this study
pXG10_ssr1528_mut	pXG10-SF	GFP fusion plasmid containing the *ssr1528* 5'UTR with mutation in the interacting region with NsiR4	Cm^R	this study
pZE12_NsiR4	pZE12-luc	Plasmid expressing NsiR4	Amp^R	this study
pZE12_NsiR4_mut1911	pZE12-luc	Plasmid expressing NsiR4 with a compensatory mutation for the mutation in *gifA*	Amp^R	this study

pZE12_NsiR4_mut1528	pZE12-luc	Plasmid expressing NsiR4 with a compensatory mutation for the mutation in *ssr1528*	Amp^R	this study

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